

Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines

PAUL H. FOURIE¹, FRANCOIS HALLEEN¹, JOHANN VAN DER VYVER² and WOUTER SCHREUDER²

¹Disease Management, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

²Hygrotech Seed, PO Box 379, Strand 7139, South Africa

Summary. The growth-stimulating attributes of *Trichoderma* treatments (dips, soil amendments and drenches with *Trichoderma* products containing propagules of selected strains of *Trichoderma harzianum*) in grapevine nurseries, and their effect on the occurrence of fungi in roots and rootstocks of nursery grapevines, in particular fungi causing Petri disease (*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.) and black foot rot (*Cylindrocarpon* spp.), were compared with quintozone/procymidone treated (standard) vines. Early shoot growth of *Trichoderma* treated vines was visibly better than that of the control vines. Eight months after planting, at uprooting, percentage take and shoot mass of *Trichoderma* and standard treated vines were similar, but total root mass was significantly higher for *Trichoderma* treated vines. Low percentages of *Cylindrocarpon* spp. were isolated from the rootstocks of treated and untreated vines, while less Petri disease fungi were isolated from rootstocks of *Trichoderma* treated vines. Markedly fewer fungi were also isolated from the roots of *Trichoderma* treated vines. Incidences of Petri disease fungi in roots of *Trichoderma* and standard treated vines were similar, but fewer *Cylindrocarpon* spp. were isolated from *Trichoderma* treated vines. These results indicate the potential of *Trichoderma* treatments in grapevine nurseries for the production of stronger vines with lower *Phaeomoniella/Phaeoacremonium* and *Cylindrocarpon* infection levels.

Key words: *Phaeomoniella*, *Phaeoacremonium*, *Cylindrocarpon*, Petri disease, black foot.

Introduction

Wine, table and raisin grape cultivars in South Africa are grafted onto rootstock cultivars to overcome certain biological (phylloxera, nematodes, fungi and bacteria), physical (poor drainage, shallow soils, etc.) and/or chemical (high or low pH,

high salinity, etc.) problems in the soil. Propagation material is taken from certified rootstock and scion mother blocks. Grafting is done during late winter to early spring, and graftlings are planted in grapevine nurseries where they grow for one season. The vines are uprooted during winter and sold to wine, table or raisin grape producers. Standard practices to prevent decay by saprophytic fungi involve the treatment of propagation material with contact fungicides (like PCNB, captan or dicarboximides) or sterilising agents (quaternary ammonium compounds) as drenches or dips at various stages before and after grafting.

The origin of two decline diseases of young grapevine in South Africa has been traced to nurseries. The causal fungi of Petri disease⁽¹⁾ (also

⁽¹⁾ At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that young grapevine decline, 'black goo', Petri vine decline will henceforth be called Petri disease.

Corresponding author: P. Fourie

Fax: +27 21 809 3002

E-mail: paul@nietvoor.agric.za

known as black goo decline), *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (Scheck *et al.*, 1998; Rego *et al.*, 2000; Groenewald *et al.*, 2001), were found to be present in grapevine rootstock propagation material, whereas the causal fungi of black foot rot, *Cylindrocarpon* spp. (Grasso and Magnano di San Lio, 1975; Sweetingham, 1983; Scheck *et al.*, 1998; Rego *et al.*, 2000), infected the roots and rootstocks of grafted vines from nursery soils (Halleen and Crous, 2001).

Trichoderma soil amendments have been heralded as beneficial to plant growth (Chang *et al.*, 1986; Windham *et al.*, 1986; Chet, 1987; Harman, 2000), antagonistic to soil pathogens (Chet, 1987; Smith *et al.*, 1990; Nemec *et al.*, 1996; Harman, 2000), and possible effectors of induced resistance (Calderon *et al.*, 1993; De Meyer *et al.*, 1998; Harman, 2000). Grapevines with better developed roots and shoots will also be better equipped to withstand stress situations, and consequently should be more resistant to stress-related diseases, in particular Petri disease (Ferreira *et al.*, 1999). The use of *Trichoderma* suspensions and soil amendments is advocated in some grapevine nurseries in Australia and New Zealand (Messina, 1999).

The purpose of this paper is to report on the growth-stimulating attributes of *Trichoderma* treatments (dips, soil amendments and drenches) in a South African nursery, and their effect on the occurrence of fungi, in particular Petri disease and black foot rot causing fungi, in the roots and rootstock of nursery grapevines.

Materials and methods

The trial was performed in a commercial nursery at Wellington in the Western Cape of South Africa. Experimental layout was a completely randomised design with two treatments and 4 repetitions. Rootstock material ('Ramsey') was dipped (5 s) in a *Trichoderma* suspension (Trichoflow-T™, selected strains of *T. harzianum*, Agrimm Technologies Ltd., Christchurch, New Zealand) directly prior to grafting. 'Sauvignon blanc' was hand-grafted to the treated rootstocks, and the graftlings were dipped in *Trichoderma* again. The standard treatments (control) consisted of dips in a mixture of quintozene (PCNB 750 WP, Plaaskem, Johannesburg, South Africa; 500 g/100 l) and procymidone (Sumislex 250 SC, Sanachem, Johannesburg,

South Africa; 200 ml/100 l) at the stages mentioned. *Trichoderma* treated and control graftlings were stacked in separate callusing boxes, covered with sawdust and stored in a callus room (8 to 15°C) for 32 days. Prior to planting, *Trichoderma* pellets (Trichopel-R™, selected strains of *T. harzianum*, Agrimm) were added to the planting furrows at 20 g m⁻¹. Control plant sites were not treated. Graftlings were planted at 5 cm spacing within rows, and 60 cm between rows. After planting, the root zones of the treated plots were drenched with *Trichoderma* (Trichogrow™, selected strains of *T. harzianum*, Agrimm) (1.5 kg ha⁻¹) at monthly intervals. A total of six applications were made. The control vines were treated with water. Normal nursery practices (irrigation, nutrition, cultural practices and disease and pest management) were followed for all the vines.

Vine growth was monitored throughout the season. The longest shoots of 27 randomly selected *Trichoderma* treated and 27 control vines were measured 5 weeks after planting. After 8 months in the nursery, the plants were uprooted and percentage take (yield of Class 1 vines as a percentage of the total number of vines planted in each plot) was determined. Twenty-five vines per replicate were randomly selected, their total root and shoot mass determined, and used for fungal isolation. Rootstock (6 cm from the basal end) and five root segments (6 cm from the basal rootstock attachment) were cut from the vines, sterilised (30 s in 70% ethanol, 2 min in 3.5% sodium hypochlorite and 30 s in 70% ethanol) and air-dried in a laminar flow cabinet. Five small (1 × 0.5 mm) sections were cut from the xylem tissue in the rootstock (approximately 3 cm from the basal end of the rootstock) and five in each root segment (3 cm from the rootstock attachment) and plated on potato dextrose agar medium (Biolab, Midrand, South Africa) amended with 250 mg l⁻¹ chloramphenicol. Inoculated plates were incubated for 21 days at 23°C under a diurnal light schedule. Fungal cultures were microscopically identified. The incidence of Petri disease fungi (*P. chlamydospora* and *Phaeoacremonium* spp.), *Cylindrocarpon* spp., "total number" of fungi and bacteria present in each rootstock and root was determined as a percentage of the five isolated segments colonised. Data were subjected to analysis of variance using SAS version 8.1 (SAS, 1990). Student's t-Least Significant

Difference was calculated at the 5% confidence level to compare treatment means.

Results

Five weeks after planting, shoots of *Trichoderma* treated vines were markedly longer than those of the control vines (102.6 ± 64.77 mm vs. 70.2 ± 35.91 mm). Eight months after planting, percentage take and shoot mass were not increased by the

Trichoderma treatments, but total root mass was significantly increased from the 25.73 g of the control vines to 36.46 g (Table 1).

The incidence of various fungal genera isolated from the rootstocks and roots of the *Trichoderma* treated and control vines is given in Table 2. *Phaeomoniella* was the more prominent of the Petri disease fungi, and colonised a mean of 4.7% of the total number of xylem segments isolated from roots and rootstocks in this trial. *Phaeoacremonium* spp.

Table 1. Mean percentage take and total root and shoot masses of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Percentage take	Total shoot mass (g)	Total root mass (g)
<i>Trichoderma</i>	$43.05 \pm 3.145a$	$20.46 \pm 0.510a$	$36.46 \pm 2.014a$
Control	$44.58 \pm 2.863a$	$18.92 \pm 2.044a$	$25.73 \pm 1.638b$
L.S.D.	10.407	5.154	6.352

Values in each column followed by the same letter do not differ significantly ($P=0.05$).

Table 2. Percentage of various fungal genera isolated from rootstocks and roots of *Trichoderma* treated and standard treated (control) nursery grapevines.

Fungal genus	Rootstock (%) ^a		Roots (%) ^a	
	<i>Trichoderma</i>	Control	<i>Trichoderma</i>	Control
<i>Acremonium</i>	1.8	1.8	1.8	1.4
<i>Alternaria</i>	0.2	0	0.2	0.2
<i>Aspergillus</i>	0	0.2	0	0.4
<i>Botryosphaeria</i>	1	0.2	0	0
<i>Chaetophoma</i>	0.4	0	0.4	1.4
<i>Cylindrocarpon</i>	0.4	0.4	1.6	2.8
<i>Cladosporium</i>	0.2	0	0	0.2
<i>Fusarium</i>	0	0.4	0.2	1
<i>Gliocladium</i>	0	0	0.2	0.2
<i>Paecilomyces</i>	0	0.2	0	0
<i>Penicillium</i>	0.2	1.2	3	1.2
<i>Phaeoacremonium</i>	1	0.8	1.2	1.4
<i>Phaeomoniella</i>	4.4	7.8	3.2	3.4
<i>Phialophora</i>	0	0	0	0.2
<i>Phoma</i>	13.6	2.8	19.2	26.6
<i>Phomopsis</i>	0.8	2.2	0	0
<i>Pyrenochaeta</i>	0	0	0.4	0
<i>Rhizoctonia</i>	0	0	0	0.2
<i>Robillarda</i>	0.2	0	0.2	0
Unidentified	0.6	2.8	1.2	3.6

^a Total number of xylem segments (5 segments per vine, 25 vines per repetition, 4 repetitions) that were colonised by the fungi, expressed as a percentage of the total number of segments used for isolation in each treatment.

colonised a mean of 1.1% of the segments, and was seemingly not influenced by the *Trichoderma* treatments. Other fungal genera that include known grapevine pathogens were *Botryosphaeria*, *Phomopsis* and *Rhizoctonia* species. *Phoma* was the most frequently isolated fungal genus (15.6%).

Statistical analyses were performed for the Petri disease fungi, *Cylindrocarpon* spp., “total fungal” and “total bacterial” counts. Due to the large variance of infection levels, a statistically significant difference at the 5% confidence level was observed for the total number of bacteria isolated from the rootstocks only (Table 3). Significantly fewer bacteria were isolated from the *Trichoderma* treated vines (23.8% vs. 37.4%). The bacteria were not identified. An equal isolation percentage of “total fungi” was obtained from rootstocks of *Trichoderma* treated and from control vines (24.8 and 25.8% respectively), as was also the case with *Cylindrocarpon* spp., which occurred at relatively low percentages (0.4% for both treatments). Although the dif-

ference was not statistically significant, markedly fewer Petri disease fungi occurred in rootstocks of *Trichoderma* treated vines (5.2 vs. 8.6%). From the roots (Table 4), none of the fungal or bacterial counts proved to be significant at the 5% confidence level, but markedly fewer bacteria and fungi were isolated from the *Trichoderma* treated vines. The incidences of Petri disease fungi in the roots of *Trichoderma* treated and control vines were similar, but fewer *Cylindrocarpon* spp. occurred in the *Trichoderma* treated vines (1.6 vs. 2.8%).

Discussion

Although the growth-stimulating attributes of *Trichoderma* soil amendments on a variety of crops have been well documented (Chang *et al.*, 1986; Windham *et al.*, 1986; Chet, 1987; Harman, 2000), this is, to the knowledge of the authors, the first report of enhanced root development of grapevine resulting from *Trichoderma* soil amendments. Al-

Table 3. Mean percentages^a of Petri disease fungi (*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.), *Cylindrocarpon* spp., total fungi and total bacteria present in rootstocks of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Microorganisms (%) ^a			
	Petri disease fungi	<i>Cylindrocarpon</i>	Total fungi	Total bacteria
<i>Trichoderma</i>	5.2 ± 1.49a	0.4 ± 0.40a	24.8 ± 3.17a	23.8 ± 2.77a
Control	8.6 ± 2.35a	0.4 ± 0.40a	25.8 ± 3.26a	37.4 ± 3.45b
L.S.D.	6.027	1.384	17.859	6.177

^a Mean percentage of xylem segments (5 segments per vine, 25 vines), that were colonised by fungi or bacteria. Values in each column followed by the same letter do not differ significantly ($P=0.05$).

Table 4. Mean percentages of Petri disease fungi (*P. chlamydospora* and *Phaeoacremonium* spp.), *Cylindrocarpon* spp., total fungi and total bacteria present in roots of *Trichoderma* treated and standard treated (control) nursery grapevines.

Treatment	Microorganisms (%) ^a			
	Petri disease fungi	<i>Cylindrocarpon</i>	Total fungi	Total bacteria
<i>Trichoderma</i>	4.4 ± 1.09a	1.6 ± 0.79a	32.8 ± 3.07a	29.4 ± 2.79a
Standard	4.6 ± 1.02a	2.8 ± 0.99a	46.2 ± 3.74a	43.4 ± 3.25a
L.S.D.	5.866	3.343	29.842	18.806

^a Mean percentage of xylem segments (five segments per vine, 25 vines), that were colonised by fungi or bacteria. Values in each column followed by the same letter do not differ significantly ($P=0.05$).

though root development was not determined at an early stage, increased root development can be anticipated from the 46.1% longer shoot growth measured 5 weeks after planting. It is common nursery practice to top vines at regular intervals to stimulate root development and prevent excessive shoot growth. As a consequence total shoot mass of the *Trichoderma* treated vines was only marginally (8.1%) better than the control vines. Nonetheless, root development of the *Trichoderma* treated vines was enhanced by 41.7%. Since a large proportion of a grapevine's reserves is stored in the roots (Hunter, 1998), this attribute would be beneficial to the vine's ability to withstand replant shock. Given the general increase in growth, it was surprising to note that the *Trichoderma* treatments did not result in better percentage take. The percentage take of both treatments was low, but nonetheless similar to what is generally experienced in South African grapevine nurseries.

However, by enhancing vine and root development, tolerance to stress would also be increased (Harman, 2000). Stress predisposes plants to attack by several pathogens, in particular, it predisposes grapevines to the Petri disease fungi, *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (Ferreira *et al.*, 1999; Fourie and Halleen, 2001), and also to *Cylindrocarpon* spp., which causes black foot rot (Scheck *et al.*, 1998; Fourie and Halleen, 2001). Almost 40% fewer Petri disease fungi were isolated from the rootstocks and 42.9% fewer *Cylindrocarpon* spp. from the roots was isolated from *Trichoderma* treated vines. These reductions were not statistically significant, but certainly remarkable, given the quiescent nature of these pathogens in these young asymptomatic vines. Besides the increased stress tolerance of *Trichoderma* treated vines, the reduced incidence of decline pathogens can also be attributed to induced resistance. *Trichoderma* has the ability to induce resistance in several other annual crops (Harman, 2000), but this phenomenon is not often reported for a perennial crop like grapevine. However, Calderon *et al.* (1993) and De Meyer *et al.* (1998) detected induced resistance responses in grapevine following inoculation with *T. viride* and *T. harzianum*, respectively.

Furthermore, by colonising the rhizosphere, *Trichoderma* might have prevented root pathogens from attacking developing roots by antagonism or

competition (Chet, 1987). The presence of *Phytophthora* and *Pythium* spp. in South African grapevine nursery soils has been shown (Marais, 1980), but because of the frequent use of phosphonates to control downy mildew in grapevine nurseries, these pathogens have not caused serious losses. Low levels of infection might however affect root reduction or slow root development. *Cylindrocarpon* spp. can also cause a reduction in root mass (Scheck *et al.*, 1998). Halleen and Crous (2001) have shown that these fungi were isolated with increasing frequency over time from roots and rootstocks of nursery grapevines. Increased root mass and general vigour, as well as the reduced incidence of *Cylindrocarpon* spp. in the roots of *Trichoderma* treated vines might therefore be attributed to biological control of these pathogens by *Trichoderma*.

Collectively, these results demonstrate the potential of *Trichoderma* treatments in grapevine nurseries for the production of stronger vines with lower *Phaeomoniella/Phaeoacremonium* and *Cylindrocarpon* infection levels.

Acknowledgements

The authors would like to acknowledge Hygrotech Seeds for partial funding of this project, and VinPro SA for partial sponsorship towards attendance of the 11th Congress of the Mediterranean Phytopathological Union and 2nd International Workshop on Grapevine Trunk Diseases.

Literature cited

- Calderon A.A., J.M. Zapata, R. Munoz, M.A. Pedreno and A.R. Barcelo, 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. *New Phytologist* 124, 455–463.
- Chang Y.-C., Y.-C. Chang, R. Baker, O. Kleifeld and I. Chet, 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Disease* 70, 145–148.
- Chet I., 1987. *Trichoderma* – Application, mode of action, and potential as biocontrol agent of soilborne plant pathogenic fungi. In: *Innovative Approaches to Plant Disease Control* (I. Chet, ed.), John Wiley & Sons, New York, NY, USA, 137–160.
- De Meyer G., J. Bigirimana, Y. Elad and M. Höfte, 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology* 104, 279–286.
- Ferreira J.H.S., P.S. van Wyk and F.J. Calitz, 1999. Slow

- dieback of grapevine in South Africa: stress-related predisposition of young vines for infection by *Phaeoacremonium chlamydosporum*. *South African Journal of Enology and Viticulture* 20(2), 43–46.
- Fourie P.H. and F. Halleen, 2001. Field observations of black goo decline and black foot disease of grapevine. In: *Abstracts, 39th Congress of the Southern African Society for Plant Pathology*, January 21–24, 2001, Nelspruit, South Africa, 35, 43 (abstract).
- Groenewald M., J. Kang, P.W. Crous and W. Gams, 2001. ITS and β -tubulin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. *Mycological Research* 105, 651–657.
- Grasso S. and G. Magnano di San Lio, 1975. Infections of *Cylindrocarpon obtusisporum* on grapevines in Sicily. *Vitis* 14, 36–39.
- Halleen F. and P.W. Crous, 2001. Fungi associated with healthy grapevine cuttings in nurseries in the Western Cape province. In: *Abstracts, 39th Congress of the Southern African Society for Plant Pathology*, January 21–24, 2001, Nelspruit, South Africa, P38, 104 (abstract).
- Harman G.E., 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease* 84, 377–393.
- Hunter J.J., 1998. Plant spacing implications for grafted grapevine. I. Soil characteristics, root growth, dry matter partitioning, dry matter composition and soil utilisation. *South African Journal of Enology and Viticulture* 19, 25–34.
- Marais P.G., 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. *Phytophylactica* 12, 9–13.
- Messina J., 1999. The use of beneficial *Trichoderma* in grapevine propagation. *Combined Proceedings of the International Plant Propagator's Society* 48, 145–148.
- Nemec S., L.E. Datnoff and J. Strandberg, 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetable and citrus. *Crop Protection* 15, 735–742.
- Rego C., H. Oliveira, A. Carvalho and A. Phillips, 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* 39, 76–79.
- SAS, 1990. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, NC, USA.
- Scheck H.J., S.J. Vasquez, D. Fogle and W.D. Gubler, 1998. Grape growers report losses to black foot and grapevine decline. *California Agriculture* 52(4), 19–23.
- Smith V.L., W.F. Wilcox and G.E. Harman, 1990. Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. *Phytopathology* 80, 880–885.
- Sweetingham M., 1983. *Studies on the nature and pathogenicity of soilborne Cylindrocarpon spp.* PhD thesis, University of Tasmania, Australia.
- Windham M.T., Y. Elad and R. Baker, 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology* 76, 518–521.

Accepted for publication: December 3, 2001